# PATENT SPECIFICATION

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## (54) METHOD FOR THE DESTRUCTION OF AQUATIC MOLLUSCS

(71) We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, Imperial Chemical House, Millbank, London SW1P 3JF, a British Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for the destruction of aquatic molluscs, particularly those molluscs which are responsible for the transmission of Bilharziasis.

Bilharziasis is a disease which is endemic in parts of Africa, the Middle East and the Far East. It is a debilitating disease and results in prolonged ill-health due to damage inflicted on various internal organs, particularly the liver. Bilharziasis is water-borne, and an essential element in its transmission is the function of certain aquatic snails as hosts for the organisms which are responsible for the disease. Human infection occurs under insanitary conditions; healthy people become infected by, for example, bathing in infected water, and water can become infected as a result of insanitary practices by infected individuals.

The disease can be attacked in three ways. Firstly, infected individuals can be treated with various drugs to eliminate the infection. Secondly, sanitary conditions can be improved in order to avoid transmission of the disease. Thirdly, the life-cycle of the organisms responsible for the disease can be interrupted by destruction of the snail hosts. It is with this last approach that the present invention is concerned.

Compounds which have previously been used as aquatic molluscicides include copper salts, especially copper sulphate, salts of pentachlorophenol, salicylic acid derivatives such as 5 - chlorosalicylic acid and dichloronitrosalicylic acid, N - tritylmorpholine and zinc dialkyldithiocarbamates.

At a concentration which is lethal to aquatic snails, copper sulphate is not harmful to man r animals. However, especially in adverse water conditions, i.e. turbid water containing much suspended matter, a concentration as high as 30 parts per million may be necessary, because some of the copper sulphate is rendered ineffective by precipitation under certain pH conditions and/or is removed by organic material. The necessity for clearing vegetation from the water to be treated thus raises the cost of the treatment. Copper sulphate is also corrosive towards metals, i.e. the pipes, tanks, pumps, etc., which are used to meter it into the water being treated. Because of the high concentration required, large quantities of the chemical have to be transported, again raising the cost of this method of treatment.

Sodium pentachlorophenate is also widely used, and at mulluscicidal strength is very toxic to snail eggs as well as to the snails themselves, although not to man or animals. It is not seriously affected by the presence of vegetation, and the concentration necessary to kill the snails in flowing water conditions is about 10 p.p.m. for 8 hours. It is cheap to use, but solid sodium pentachlorophenate is a powerful irritant, and both solid and concentrated aqueous solutions thereof are highly toxic, so that adequate handling precautions must be taken.

Copper pentachlorophenate, prepared *in situ* by mixing copper sulphate and sodium pentachlorophenate in the water to be treated, is essentially insoluble in water and is consequently much more persistent than either copper sulphate or sodium pentachlorophenate individually. It is an effective molluscicide, but the unpleasant affects of handling toxic chemicals have still to be considered and precautions taken.

5 - chlorosalicylic acid is lethal to aquatic snails at about 1 p.p.m. and also kills snails' eggs, but is not toxic to mammals at this concentration. It is sparingly soluble in water. Undesirable features are that it may scorch vegetation and destroy algae. The compound is destroyed by the action of ultra-violet light.

All the molluscicides in current use have certain disadvantages and at their effective

concentrations as molluscicides may als have an adverse effect on desirable aquatic life such as vegetation and fish.

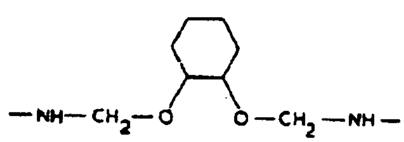
It has now been found that certain polymeric biguanides are effective aquatic molluscicides.

According to the present invention there is provided a method for the destruction of aquatic molluscs and/or their eggs in water infested therewith which comprises adding to the aquatic habitat of the molluscs and/or their eggs to provide a concentration from 0.1 to 100 parts per million, a linear polymeric biguanide or a salt thereof which in its free base form has a recurring polymer unit represented by the formula

$$-X-NH-\underset{\substack{\| \\ NH}}{C}-NH-\underset{\substack{\| \\ NH}}{C}-NH-Y-NH-\underset{\substack{\| \\ NH}}{C}-NH-\underset{\substack{\| \\ NH}}{C}-NH-$$

wherein X and Y represent bridging groups in which together the total number of carbons atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is more than 9 and less than 17.

The bridging groups X and Y may consist of polymethylene chains, optionally interrupted by hetero atoms, for example, oxygen, sulphur or nitrogen. X and Y may also incorporate cyclic nuclei which may be saturated or unsaturated, in which case the number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is taken as including that segment of the cyclic group, or groups, which is the shortest. Thus, the number of carbon atoms directly interposed between the nitrogen atoms in the group



is 4 and not 8.

The preferred polymeric biguanide for use in the present invention is poly(hexamethylene biguanide), in which X and Y both represent the $-(CH_2)_6$-group. The compound is preferably employed in the form of its hydrochloride salt, which is conveniently used as a 20% w/w aqueous solution (i.e. 100 parts by weight of the solution contain 20 parts by weight of the active agent). Other salts of polymeric biguanides using organic and inorganic acids may be used, for example, the sulphate, acetate, gluconate or behenate.

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Polymeric biguanides may be prepared by the reaction of a bisdicyandiamide having the formula

$$CN-NH-\underset{\substack{\| \\ NH}}{C}-NH-X-NH-\underset{\substack{\| \\ NH}}{C}-NH-CN$$

with a diamine $H_2N-Y-NH_2$, wherein X and Y have the meanings defined above; or by reaction between a diamine salt of dicyanimide having the formula

$$(H_3\overset{+}{N}-X-\overset{+}{N}H_3)\,(\overset{-}{N}(CN)_2)_2$$

with a diamine $H_2N-Y-NH_2$ wherein X and Y have the meanings defined above. These methods of preparation are described in U.K. Specifications Nos. 702268 and 1152243 respectively, and any of the polymeric biguanides described therein may be used according to the present invention.

The polymer chains are terminated either by an amino group or salt thereof or by an

$$-NH-\underset{\substack{\| \\ NH}}{C}-NH-CN$$

group, and the terminating groups may be the same or different on each polymer chain.

A small proportion of the primary amine $R-NH_2$—, where R represents an alkyl group containing from 1 to 18 carbon atoms, may be included with the diamine $H_2N-Y-NH_2$ in the preparation of polymeric biguanides as described above. The primary monoamine acts as a chain-terminating agent and consequently one or both ends of the polymeric biguanide polymer chains may be terminated by an —NHR group. The use of these chain-stopped polymeric biguanides is also within the scope of the present invention.

It is to be understood that by either of the above-described methods the polymeric biguanides are obtained as mixtures of polymers in which the polymer chains are of different lengths, the number of individual biguanide units, i.e.

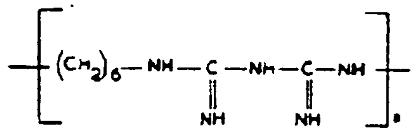
$$-X-NH-\underset{\substack{\| \\ NH}}{C}-NH-\underset{\substack{\| \\ NH}}{C}-NH-$$

and

$$-Y-NH-\underset{\substack{\| \\ NH}}{C}-NH-\underset{\substack{\| \\ NH}}{C}-NH-$$

together being from 3 to about 80.

In the case of the preferred poly(hexamethylene biguanide) having the formula



the value of n is in the range from 6 to 10, the average molecular weight of the polymer mixture being from about 1100 to about 1800.

Polymeric biguanides as defined above are in general effective as molluscicides at a concentration of approximately 1 p.p.m. At this concentration the compounds have very low toxicity towards other aquatic life and are non-toxic to animals and man. A somewhat higher concentration may be necessary in turbid water.

The invention is illustrated but not limited by the following Example in which parts and percentages are by weight.

### Example

$LC_{50}$ values for adult snails and egg masses were estimated by exposing the animals for 24 hours to various dilutions of the polymeric biguanide made up in 10° AHW (artificial hard water containing 0.104 g. calcium chloride and 0.26 g. magnesium sulphate heptahydrate per litre of distilled water). In all tests, snails and egg masses were kept singly in 200 ml. volumes of 10° AHW the temperature was 26°C throughout. After the exposure period, the animals were rinsed with 10° Artificial Hard Water (AHW) and then held in 10° AHW for a further 48 hour recovery period after which the numbers dead and alive are counted.

Using the above method the $LC_{50}$ of poly-(hexamethylene biguanide) hydrochloride against *Biomphalaria glabrata* was approximately 2.0 p.p.m. for the adult snail (10 mm shell diameter) and 0.25—0.5 p.p.m. for young, immature snails (2 mm shell diameter). Tests at pH 5.5 and 7.5 showed the compound to be slightly more active at the higher pH.

the expousre period of the snails t the biocide solution was extended to 4 days, renewing the test solution each day. Dosages of 2, 1, 0.5, 0.25, 0.1 and 0.05 p.p.m. of poly(hexamethylene biguanide) hydrochloride were used. The $LC_{50}$ for adult snails after 4 days was 0.25—0.5 p.p.m.

For 1—2 day old egg masses (the eggs hatch in 6—7 days under laboratory conditions) 20 p.p.m. of poly(hexamethylene biguanide) hydrochloride were required to kill embryos using a 24 hour exposure. However, with a 4 day exposure, the control groups and those exposed to 0.1 and 0.05 p.p.m. of the biocide hatched when they were 7 days old. With higher concentrations, the following results were obtained:

2.0 p.p.m.—all embryos died in the egg mass within 11 days after the treatment period.

1.0 p.p.m.—all embryos died in the egg mass within 21 days after the treatment period.

0.5 p.p.m.—some hatchings occurred during the 21 day recovery period but the hatchlings did not appear to feed and eventually died.

0.25 p.p.m.—though hatching was delayed, about 75% of the embryos had hatched after 21 days and these fed and began growing.

It is clear from the above results that as little as 0.5 p.p.m. of poly(hexamethylene biguanide) hydrochloride is effective in killing the embryos of *Biomphalaria glabrata* after a 4 day exposure period.

WHAT WE CLAIM IS:—

1. A method for the destruction of aquatic molluscs and/or their eggs in water infested therewith which comprises adding to the aquatic habitat of the molluscs and/or their eggs to provide a concentration from 0.1 to 100 parts per million by weight, a linear polymeric biguanide or a salt thereof which in its free base form has a recurring polymer unit represented by the formula:

$$-X-NH-\underset{\underset{NH}{\|}}{C}-NH-\underset{\underset{NH}{\|}}{C}-NH-Y-NH-\underset{\underset{NH}{\|}}{C}-NH-\underset{\underset{NH}{\|}}{C}-NH-$$

wherein X and Y represent bridging groups in which together the total number of carbons atoms directly interposed, as hereinbefore defined, between the pairs of nitrogen atoms linked by X and Y is more than 9 and less than 17.

2. A method as claimed in claim 1 wherein the polymeric biguanide is poly(hexamethylene biguanide) hydrochloride.

3. A method as claimed in claim 1 or claim 2 wherein the polymeric biguanide is employed at a concentration of approximately 1 part per million.

4. A method for the destruction of aquatic

molluscs and/or their eggs in water infested therewith, substantially as hereinbefore described with reference to the foregoing Example.

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